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X 3

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MANZ et al

Serial No. 10/046,564

Filed: January 16, 2002

For: FLUID TRANSPORT APPARATUS AND METHOD



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March 27, 2002

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0101118.8

UK

16 January 2001

Respectfully submitted,

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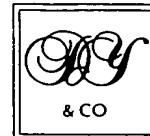
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1. Your reference **16 JAN 2001** P010665GB KMB

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17JAN01 E598432-5 D02246
P017700 0.00-0101118.8

3. Full name, address and postcode of the
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Patents ADP number (if you know it)

If the applicant is a corporate body, give
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14050746001

4. Title of the invention Fluid Transport System

5. Name of your agent (if you have one)

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FLUID TRANSPORT SYSTEM

The present invention relates to a fluid transport system, in particular a chromatographic system.

5

Chromatographic systems have seen tremendous development in recent years, but there is still a requirement for a micro-fabricated chip-based system which utilises minimal fluid volumes and provides for rapid separation of the analytes.

10 It is an aim of the present invention to provide an improved fluid transport system, in particular an improved chromatographic system.

Accordingly, the present invention provides a fluid transport system, comprising: a transport channel including a fluid inlet; and an evaporator including at least one
15 channel each having at least one open fluid outlet, for evaporating fluid at the at least one fluid outlet and causing the flow of fluid through the transport channel.

Preferably, the transport system is a chromatographic system and the transport channel includes a separation channel.

20

Preferably, the evaporator includes a gas conditioner for conditioning the gas at the at least one fluid outlet.

Preferably, the gas conditioner comprises a gas flow unit for maintaining a gas flow
25 over the at least one fluid outlet.

Preferably, the evaporator includes a heater for raising the temperature at the at least one fluid outlet.

30 Preferably, the evaporator includes a plurality of fluid outlets.

Preferably, at least one of the at least one channel of the evaporator is branched.

Preferably, the evaporator includes a plurality of channels.

- 5 In a preferred embodiment the present invention is directed to a high performance/pressure liquid chromatography (HPLC) system.

In contrast to other analysis techniques, such as capillary electrophoresis (CE) (1, 2), HPLC has not yet been achieved on a substrate chip. Previous work by several groups
10 has demonstrated the difficulty in applying a sufficiently high pressure to the channels in the chip (3), and all these attempts utilized an external pump to create the driving pressure.

The present invention advantageously provides a fluid transport system in which the
15 pressure is generated within the transport channel.

One example in nature of such a process is the transpiration of water in plants. Water transport in plants puzzled scientists for more than a hundred years. In 1895 Dixon and Joly created the "The Cohesion Theory" (4). In this theory, water transport
20 laterally and vertically is modelled only by tension created in the vessels due to transpirational pull. This theory requires that plants be able to produce enough negative pressure to counterbalance the atmospheric pressure. This means that plants like *Sequoia gigantea*, which can grow up to 100 m, should be able to produce a negative pressure of about -1 MPa. Several studies have been undertaken to prove this
25 theory, but failed due to questionable equipment. In the mid 1990s U. Zimmermann introduced a direct turgor pressure measurement with an adequate pressure sensor (5). According to his results, he established a new theory, which explains that the water transport in plants results from a combination of several forces. The "Multi Force Theory" involves the combined effects of *inter alia* transpiration driven tension, tissue
30 osmotic pressure, and gel-filamentous compounds (6). The transpiration on the surface of leaves can be described as follows. The water principally evaporates in the stomata over the cell membranes of the parenchyma tissue cells. This evaporation

leads to an increase in the concentrations of solutes in the cytoplasm, with an influx of water from adjacent cells counterbalancing this effect. The xylem, a capillary pipeline tissue, releases the water for these parenchyma cells through the mechanisms mentioned above, and also transmits negative pressure, resulting from the loss of water, towards the roots.

In a capillary channel, liquid therein is moved by the capillary force towards the gas/liquid interface, where the liquid evaporates continuously, due to the liquid vapour pressure. This lowers the volume of liquid infinitesimally and is counterbalanced by refilling of the capillary through capillary force. Important factors are that the capillary force essentially depends on the contact angle between the liquid and inner capillary wall, the circumferences of the channels and the ability of the liquid to transmit the pressure differential. Unlike the channel geometry, the contact angle and the viscosity are material properties, which cannot be altered for a given system. As illustrated in Figure 1, in order to enhance the capillary force, the circumference may be scaled up for a given cross section by changing the shape of the channel.

The capillary force is described as:

$$F_{\text{Capillary}} = \sigma \cdot l$$

σ is the surface tension

l is the length of the circumference

Therefore, the channel geometry is of significant importance. For maximum capillary force, the circumference has to be increased for a given channel cross section. Since wide and shallow channels have a tendency to collapse, the determination of an optimal channel geometry is quite complex. Channels of a small scale, typically a few micrometers, can be produced quite readily and are stable in height.

Furthermore, the concept of open tubular (OT) channels is ideal for channels of very small dimension, and, in a preferred embodiment, the present invention is directed to an open tubular-HPLC (OT-HPLC) system.

- 5 The efficiency of chromatographic systems depends on several parameters, such as the column geometry (length and inner diameter), column material, applied driving force, etc. In the 1950s, Aris and Golay founded the basic equations, which describe open tube chromatography (OTC). In comparison to a packed column, an open tubular system has at most a coating on the inner capillary wall. According to theory,
- 10 channels with very small dimensions are ideally suited for open tube chromatography and efficiencies in excess of 1 million theoretical plates can be achieved (7). One of the first attempts to run OT-HPLC on chip was ten years ago, the so called "Hitachi-chip" (8).
- 15 Preferred embodiments of the present invention will now be described hereinbelow by way of example only with reference to the accompanying drawings, in which:

Figure 2 illustrates a micro-fabricated liquid transport system in accordance with a first embodiment of the present invention. The micro-fabricated chip-based fluid transport

20 system 1 includes a liquid inlet 2, in this embodiment a three-point inlet, a transport channel 3, in this embodiment a separation channel or column, and an evaporator 5.

Figure 3 illustrates the fluid transport system 1 of Figure 1 mounted in a chip holder 6. In this embodiment the evaporator 5 includes a fan 8 for maintaining an air flow over

25 the fluid outlets of the evaporator 5 and conditioning the air thereat.

Figure 4 illustrates a micro-fabricated liquid transport system in accordance with a second embodiment of the present invention. The micro-fabricated chip-based fluid transport system 1 includes a liquid inlet 2, in this embodiment a two-point inlet, a

30 transport channel 3, in this embodiment a separation channel or column, and an evaporator 5.

Figures 5(a) to (d) illustrate preferred inlet configurations.

Figures 6(a) to (d) illustrate preferred separation channel configurations.

5 Figures 7(a) and (b) illustrate preferred single channel evaporator configurations.

Figures 8(a) to (d) illustrate preferred multi-channel configurations.

Figure 9 illustrates a chip design in accordance with a first preferred embodiment.
10

Figure 10 illustrates a chip design in accordance with a second preferred embodiment.

Figure 11 illustrates a chip design in accordance with a third preferred embodiment.

15 Figure 12 illustrates a chip design in accordance with a fourth preferred embodiment.

Figure 13 illustrates a chip design in accordance with a fifth preferred embodiment.

Figures 14(a) and (b) illustrate a chip design in accordance with a sixth preferred
20 embodiment.

Figures 15(a) and (b) illustrate a chip design in accordance with a seventh preferred
embodiment.

25 Figures 16(a) and (b) illustrate a chip design in accordance with an eighth preferred
embodiment.

Figures 17(a) and (b) illustrate a chip design in accordance with a ninth preferred
embodiment.

30

In a preferred embodiment the micro-fabricated devices are fabricated using the direct-write laser lithography process. This process can be used to etch large, complex

structures, typically up to around 10 x 10 cm, with very narrow channels, typically a few microns, in glass or silicon without the need for a mask. The process can also be used to create moulds for polymer devices, for example in PDMS, and masks which can be used for more conventional forms of lithography. As illustrated in Figure 18,

5 the micro-fabrication process is as follows:

- 10 a) The first step in the micro-fabrication of a device is to design the chip layout using a CAD package, such as AutoCAD. This design is then converted into the machine data format used by the lithography system via special conversion software.
- b) A direct-writing laser exposes a commercially available glass substrate/wafer, which contains a metal layer and photo resist.
- c) After exposure, the substrate is then developed to remove the exposed photo-resist, leaving an image of the design in the photo-resist.
- 15 d) The metal layer is then etched away using a suitable etching solution to reveal the glass.
- e) The glass is then etched to produce the channels of the device. The amounts of hydrofluoric acid (HF) and ammonium fluoride (NH_4F) for glass etching differ depending on the required etch rate.
- 20 f) After etching, the photo-resist and any metal layer are removed and a glass cover-plate is thermally bonded on top of the substrate to complete the device. Holes are drilled in the cover-plate before bonding to interface the device with the necessary pumps and injection systems.

25 Example 1

In order to enable visualisation of the movement of the liquid in the fluid transport system of Figure 14, polybeads of 10 μm in diameter were introduced into the channel 3. Measurements were obtained by estimating the travel time for a given distance (see

30 Figure 19). The velocity of the liquid within the device for 10 μm beads was $\geq 350 \mu\text{m/s}$. The measurements show little difference in the velocity regardless of air condition. However, significantly, with no air conditioning, some beads changed their

velocity (increasing as well as decreasing) occasionally by a significant amount. Moreover, it has been established that the liquid velocity is due to the evaporation and not height effects.

5 Example 2

Fluorescence experiments were also been performed. Fluorescein (5 mM in sodium phosphate buffer pH 8.08) was passed through the channel 3 from the inlet. These experiments show smearing at the inlet cross-section into the channel 3. Modified
10 inlets have been developed, including the inlet of Figure 5(c) in which the sample is injected from first to second angled inlets by an electro-generated current.

In a preferred embodiment liquids other than water, such as acetonitrile and methanol, can be used as the operating medium.

15

In preferred embodiments the channels of the transport system have sub-micron dimensions. In terms of lithographic techniques, channel widths of less than 1 μm can be achieved. There is an upper limit to the channel width where the channel is unsupported, as, if the channels are too wide, then the upper substrate layer can deform
20 or fracture. Therefore, wide channels require support structures. The channel depth is limited by the overall substrate thickness. The channel length is for practical purposes not limited as channels having a 30 m meander can be achieved even on a small chip. Generally, the longer the channel, the greater the separation, the longer the travel times and the greater the band broadening. Also, the longer the channel, the higher the
25 backpressure created therewithin. This said, although pumps capable of generating over 400 bar are available, those pumps cannot be readily coupled to a chip. For channels with sub-micron dimensions in either depth or width, the pressures required to transport liquid therethrough can easily be several thousand bar. These pressures are not obtainable in pumped systems and can only be achieved by the evaporative
30 system of the present invention.

The preferred channel geometries are as follows:

Length [cm]	
1-3	Suitable for CE and synthesis chips, very fast
5-30	Suitable for CE, synthesis and chromatography chips, still fast
30-100	Suitable for GC and LC chips
100-500	Suitable for GC and LC chips
500-3000	LC chips

Width [μm]	
0.2-2	Suitable for inlets or in splitter areas as part of a mixing device
2-10	Suitable for inlets and small separation channels, high pressure being required to transport the liquid through long channels
10-50	Suitable for all devices
50-200	Suitable for all devices
200-1000	Where shallow, two-dimensional analysis can be performed

5

Depth [μm]	
0.1-1	Suitable for open tubular chromatography, allows fabrication from silicon with a glass cover plate
1-10	Suitable for all applications
10-50	Suitable for all applications
50-250	Suitable for biological applications
250-1000	Suitable for high volume applications

CE = Capillary Electrophoresis

10 GC = Gas Chromatography

LC = Liquid Chromatography

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- 10 (5) Balling, A.; Zimmermann, U. *Planta* **1990**, *182*, 325-338.
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- 15 (8) Manz, A.; Miyahara, Y.; Miura, J.; Watanabe, Y.; Miyagi, H.; Sato, K., *Sensors and Actuators B-Chemical* **1990**, *1*, 249-255.

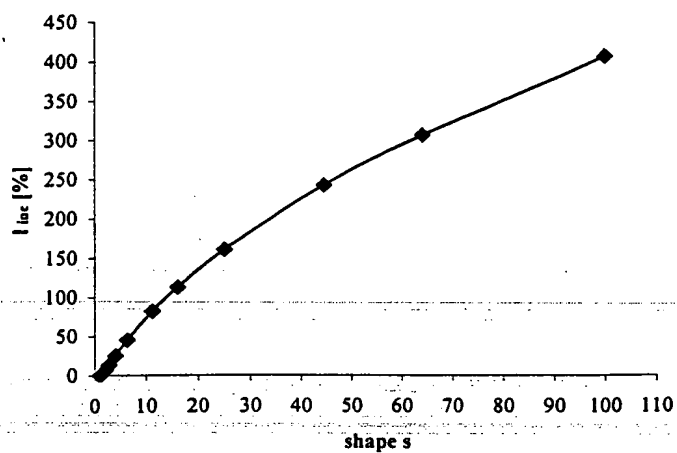


Fig.1 The increase in circumference is dependent on the relation between width vs. depth of a channel ($s = \text{width/depth}$). The cross section is constant. For example: A channel, which is 10 times wider than deep has an increase of more than 50% capillary force in comparison to a square one.

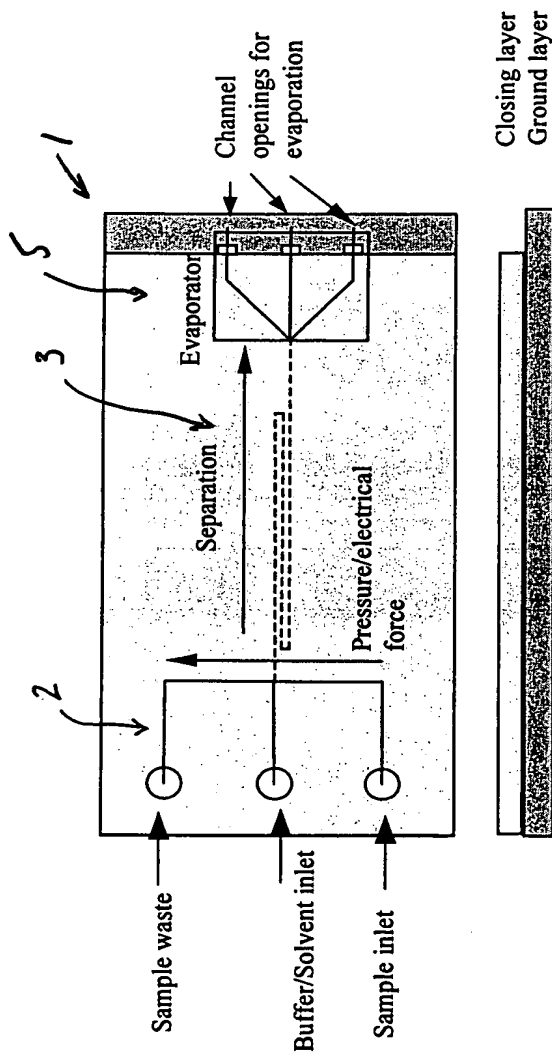


Fig. 2

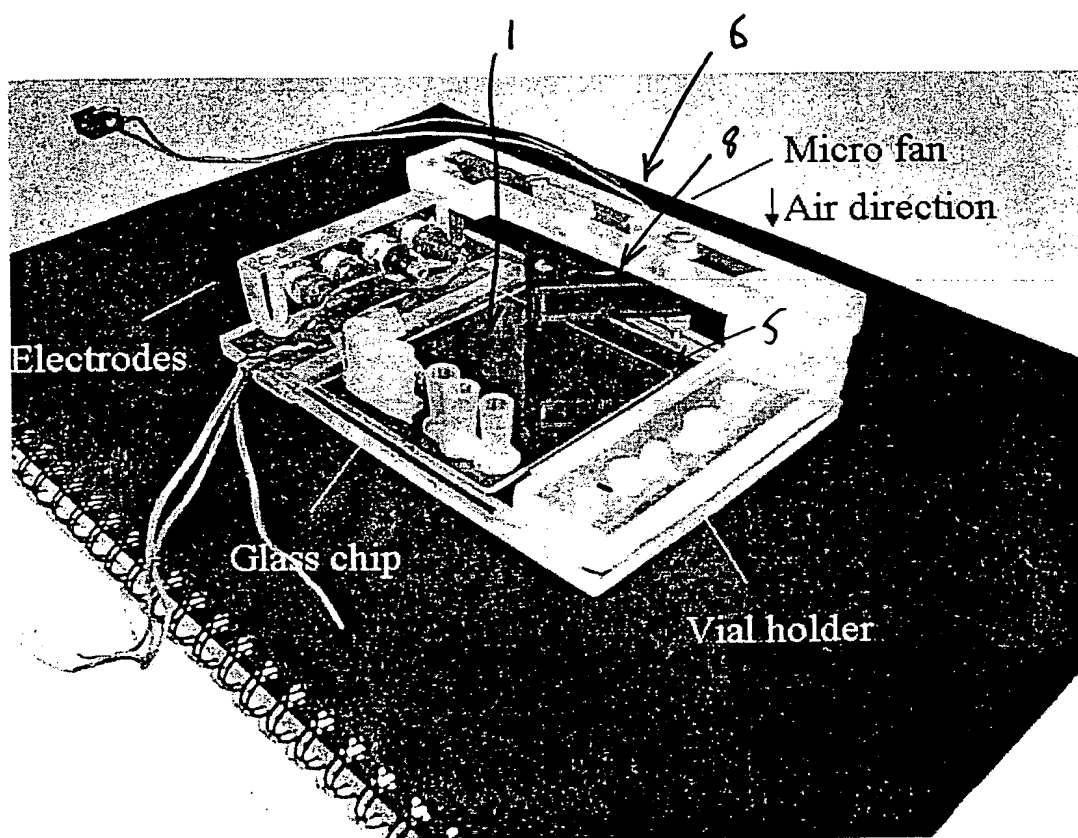


Fig. 3 Chip holder for 3in x 3in glass chips compatible with standard microscope stages; includes a micro fan for constant "fresh" air, vial holders and electrodes for sample injection

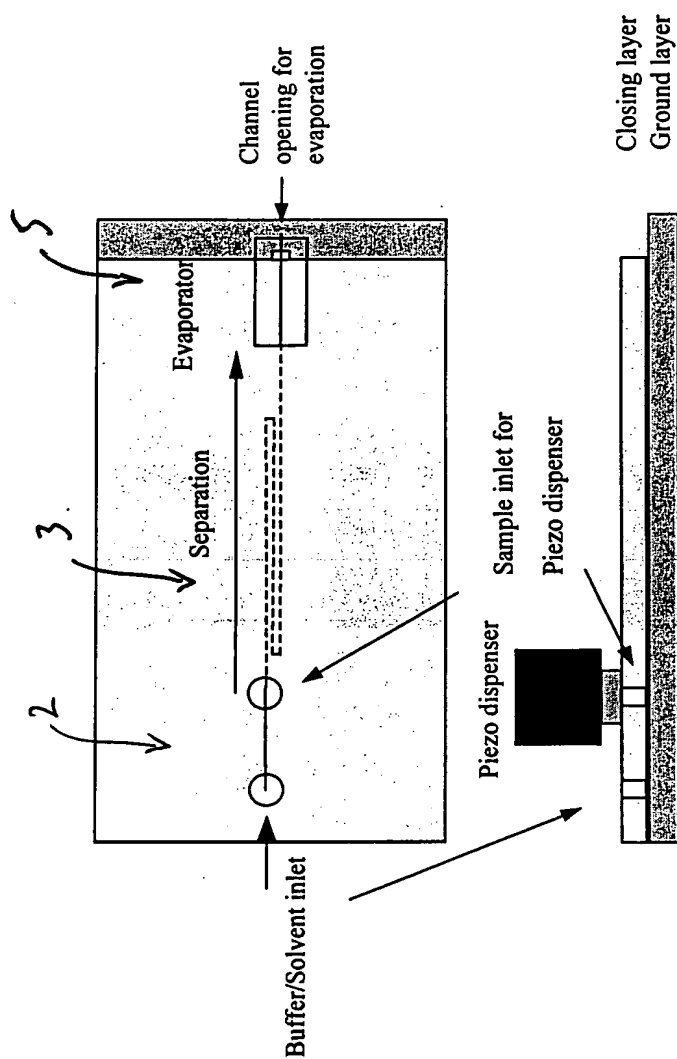
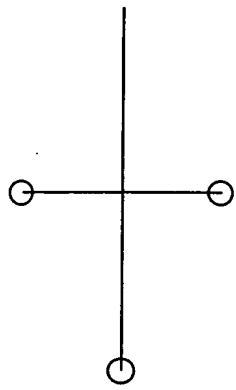


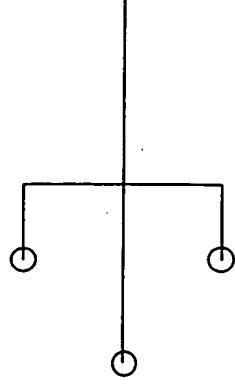
fig. 4

Inlets



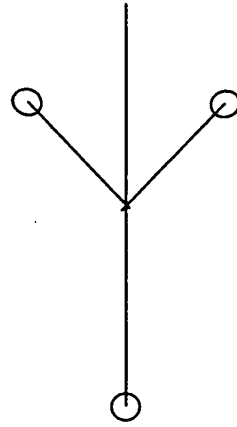
T-Inlet classic

fig. 5(a)



T-Inlet, modified

fig. 5(b)



T-Inlet, anti-stream

fig. 5(c)

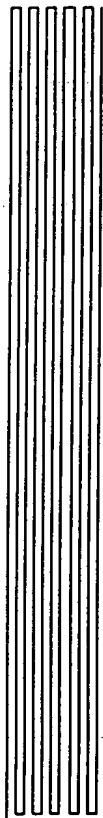


Inject-Inlet

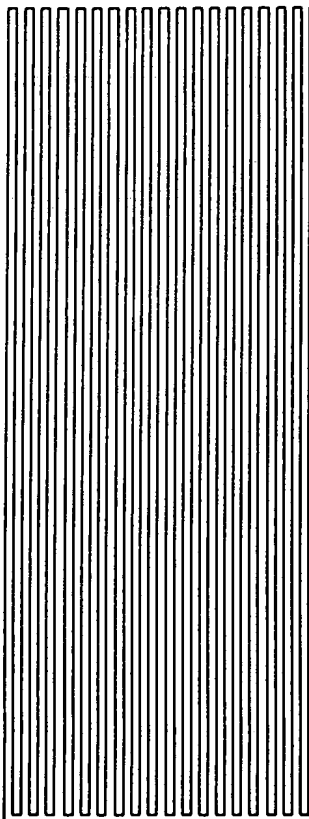
fig. 5(d)

Separation Channel

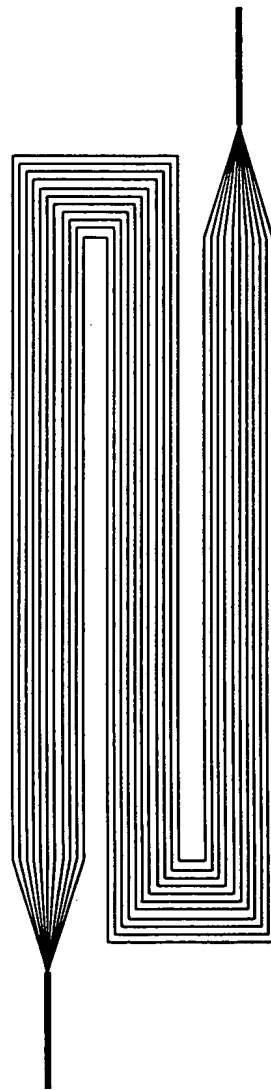
Single channel straight



Single channel meander



Single channel meander extra long



Channel bundle parallel, meander

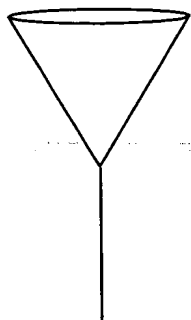
Fig. 6(d)

Fig. 6(a)

Fig. 6(b)

Fig. 6(c)

Evaporators



Funnel-shape

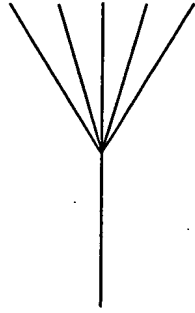
fig 7(b)



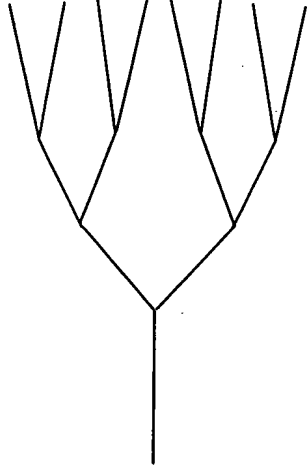
Single channel

fig 7(a)

Multi Channel Evaporators



Umbel-Shape Fig. 8(a)



Root-Shape Fig. 8(b)

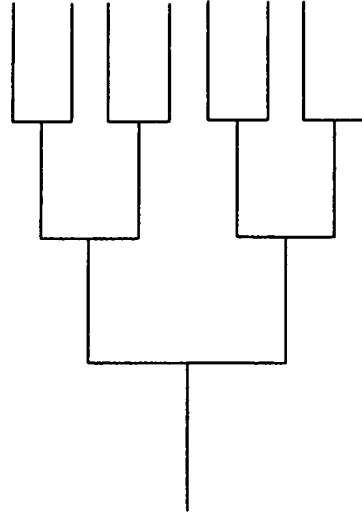


Fig. 8(c)

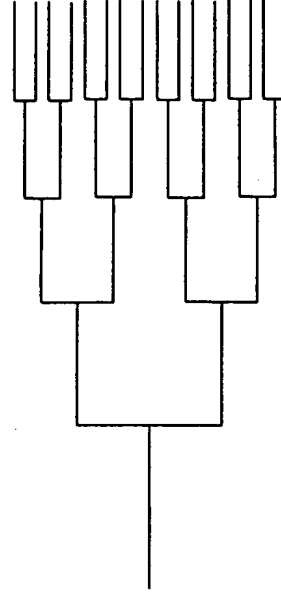
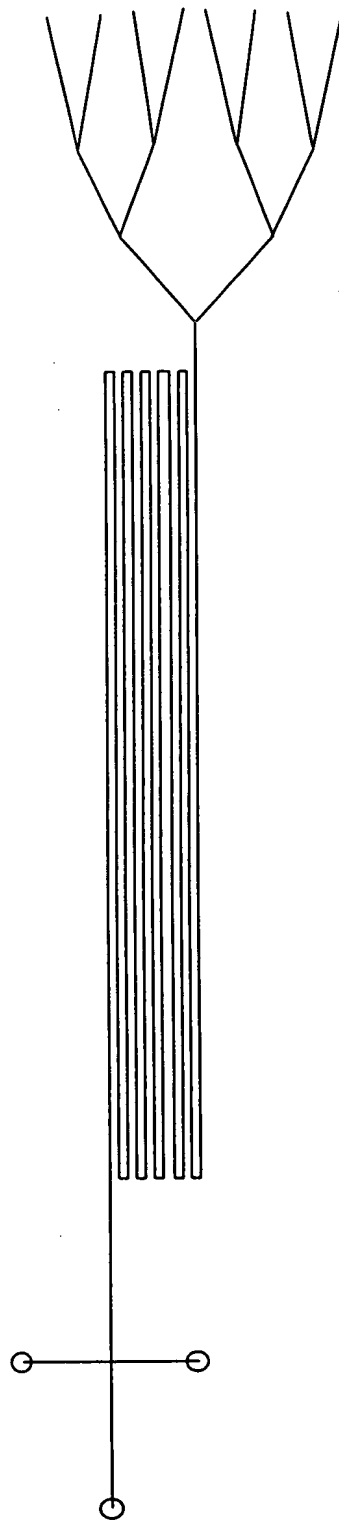


Fig. 8(d)

1:1 Splitter, rectangular 3-fold

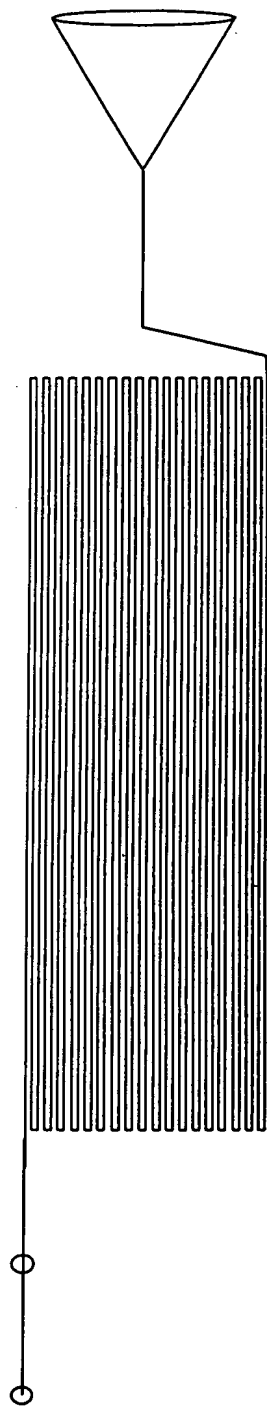
1:1 splitter, rectangular 4-fold

Fig 9'

Chip design with classic T-inlet and medium length meander single channel Separator including multi channel root-shape evaporator; all channel dimensions are the same (10 μm wide and 0.5 μm deep)

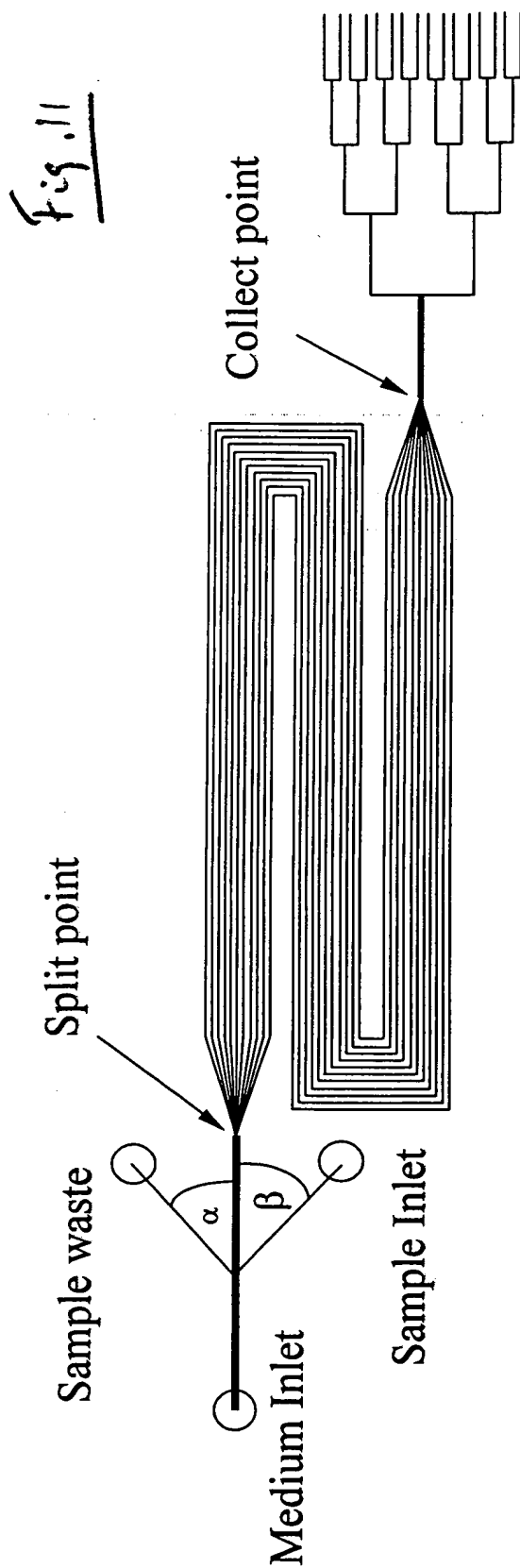
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Fig. 10.

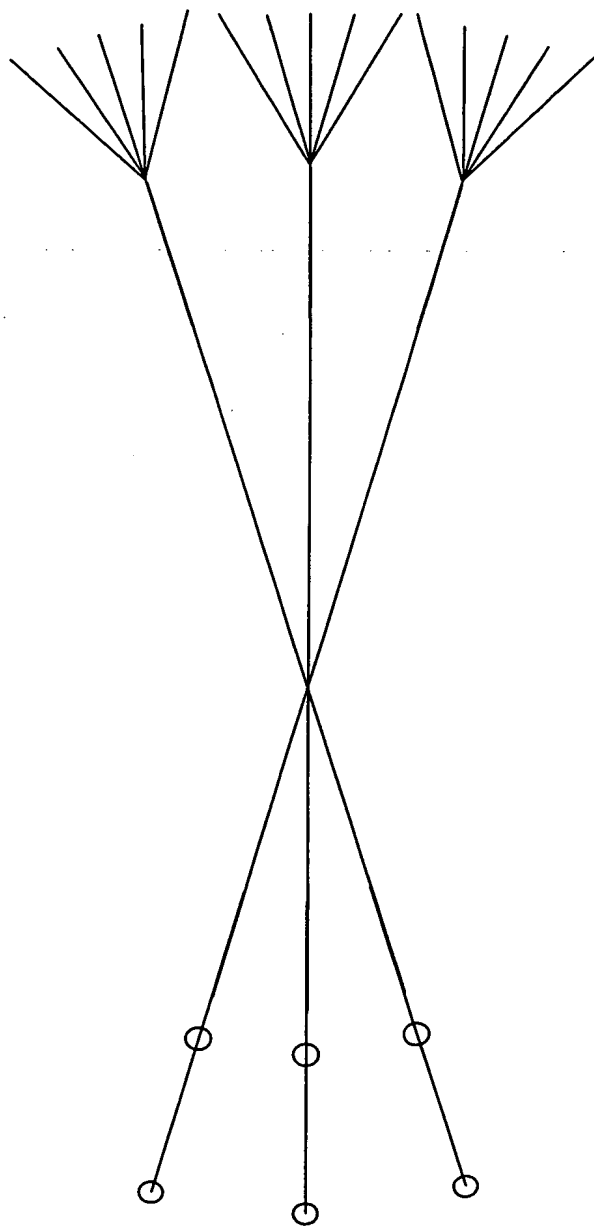


Chip design with inject-inlet including extra long single meander channel for separation.; funnel-evaporator



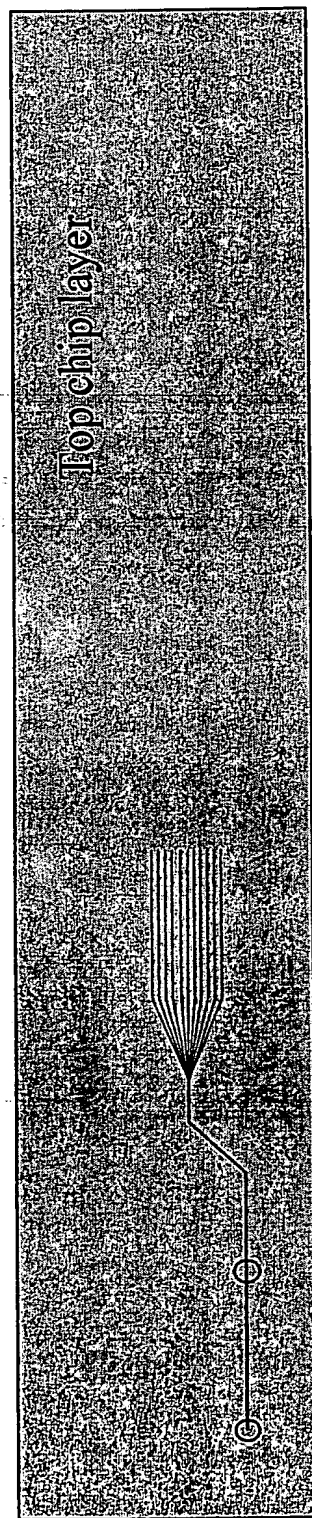
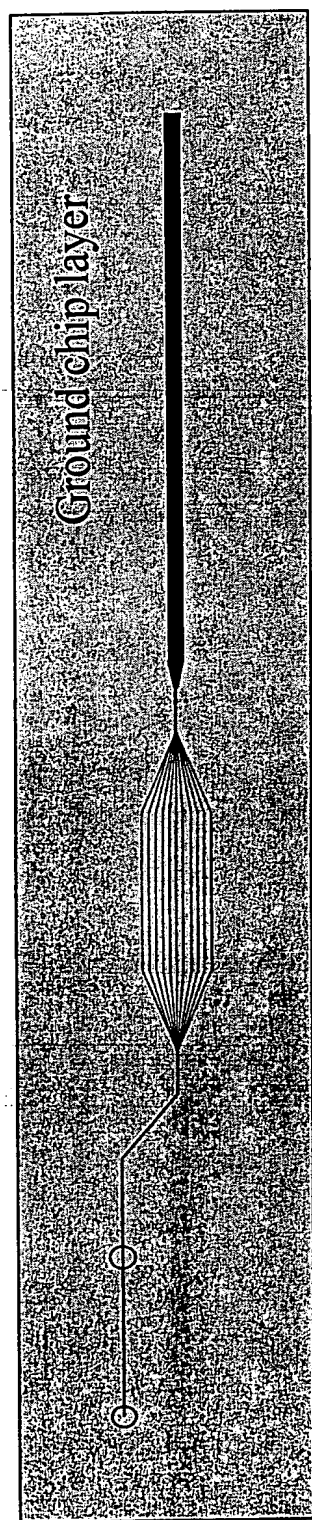


Chip design including an anti-stream inlet with different angles (α , β) for sample inlet and sample waste, channel dimensions vary between the different regions; bundle of 11 separation channels meandering parallel; evaporator 4-fold 1:1 splitter



Chip design for a three compound synthesis including three umbel-shape evaporators and three inject-inlets

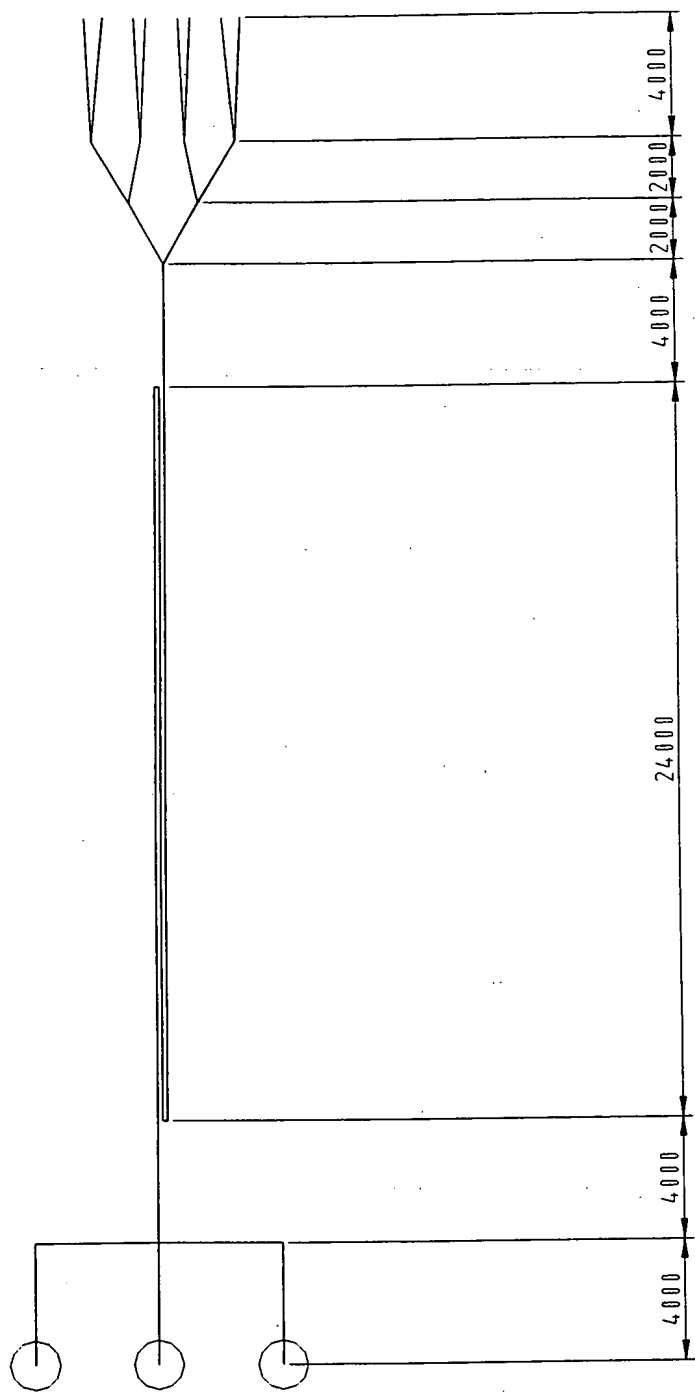
Fig. 12



Chip design for Immuno-assays including two inject-inlets on two different layers and following "Bessoth-mixer" (Lit); single wide channel evaporator

Fig. 13





design pop02, created 04-04-2000 @ Nils Goedecke

Channel width 110µm after etching, depth 25 µm over the whole structure

Fig. 14(a)

15/23

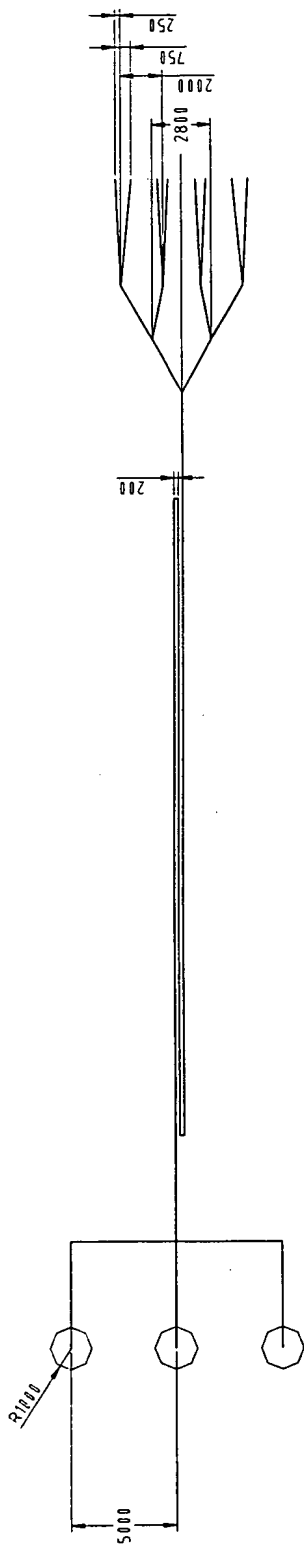
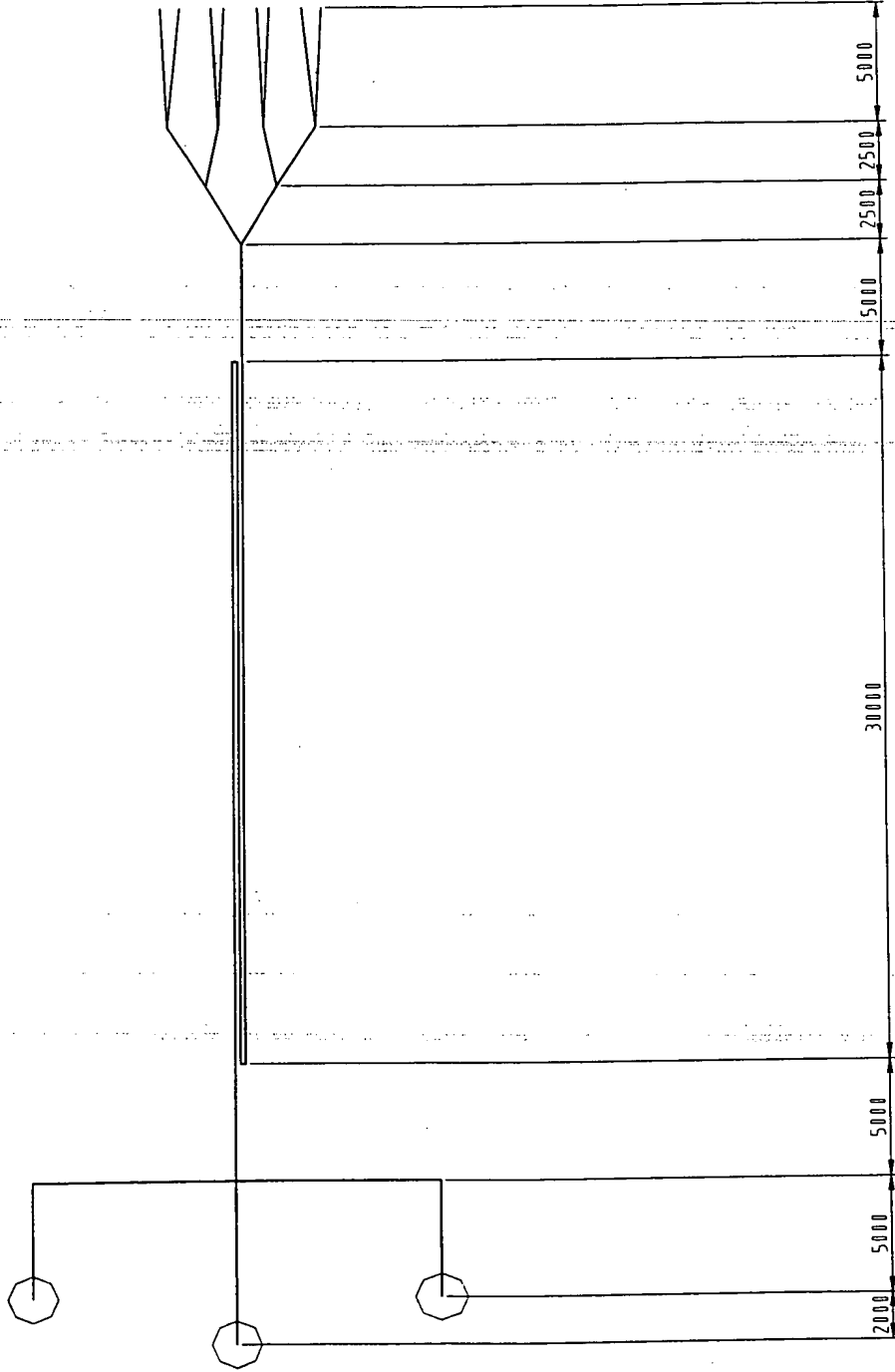


fig. 14 (b)

design pop02, created 04-04-2000 @ Nils Goedecke

16/23

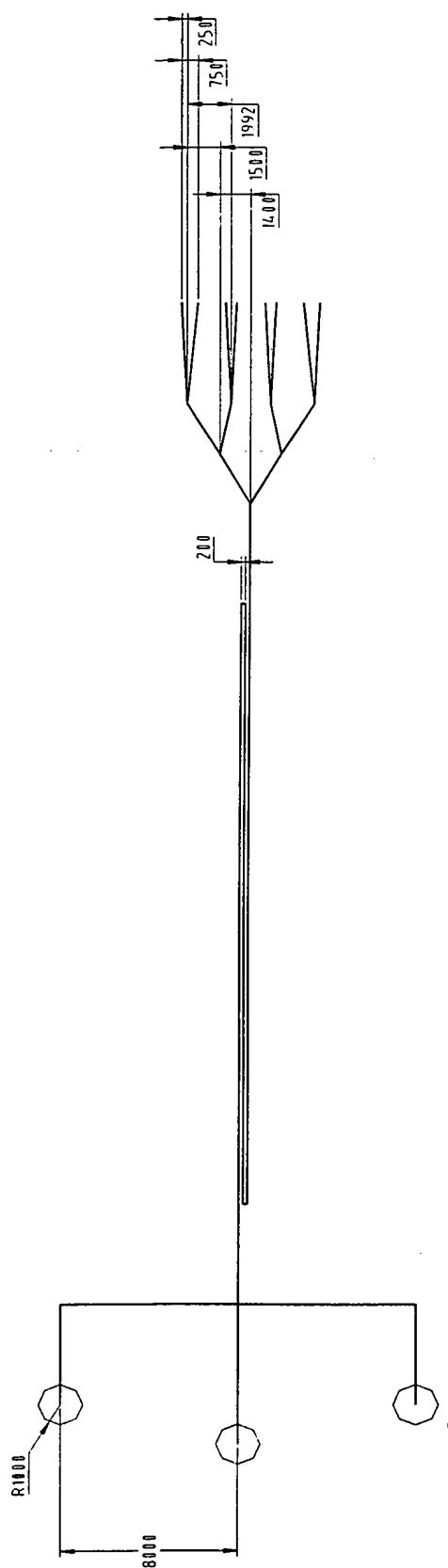
Fig 15(a)



channel width 40 microns for each design

design pop03a by Nils Goedecke 23. June 2000 IC Department of Chemistry

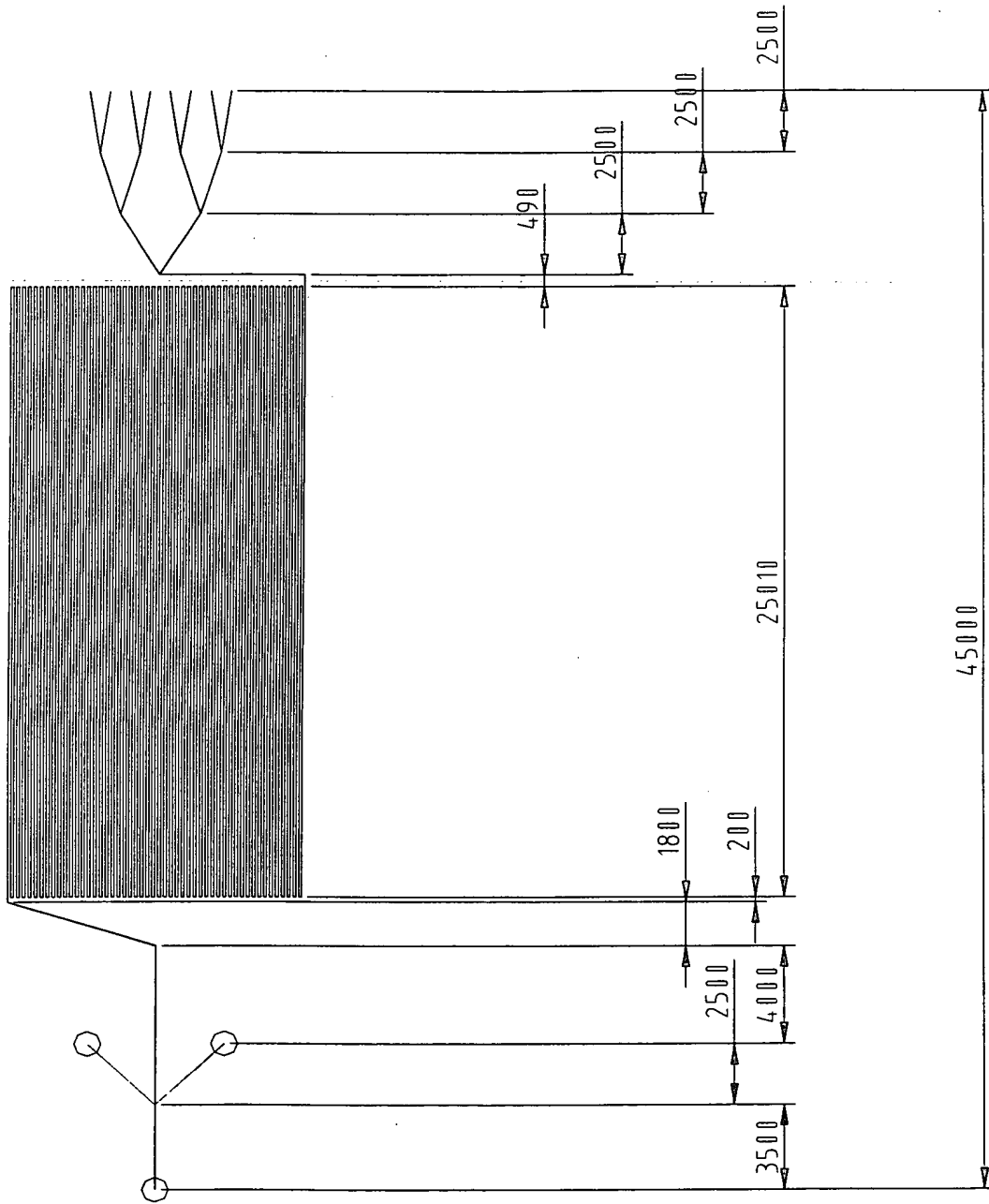
Channel width after etching 60 μ m; depth 10 μ m



channel width 40 microns for each design

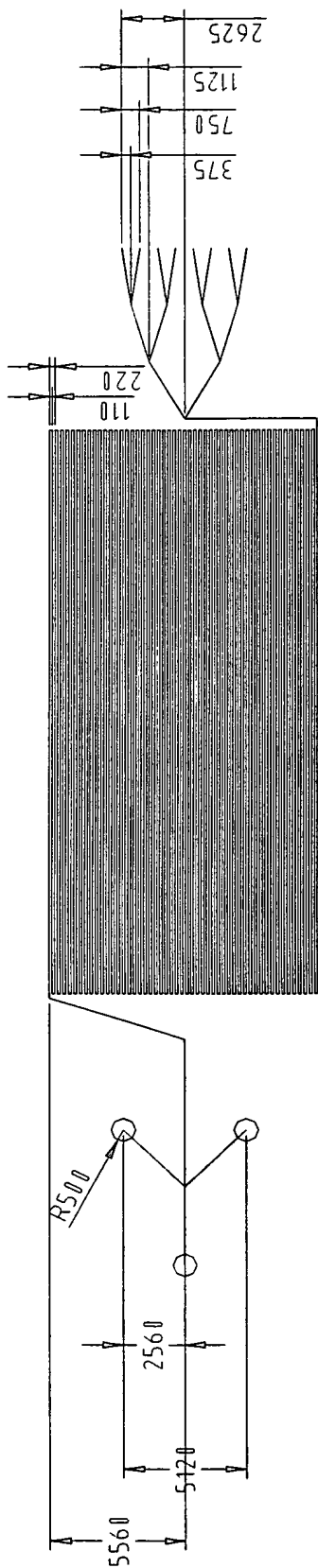
design pop03a by Nils Goedecke 23. June 2000 IC Department of Chemistry

Fig. 15(b)



Design lim 01: S I 5; Sep Ch W 10; EVvap Ch W 10 by Nils Goedecke 05.07.2000

Fig. 16 (a)



This layout includes the anti-stream-inlet and a 2.5m separation channel. Theoretically, a channel of this length 10 μ m wide and 0.1 μ m deep if running with a $\eta \sim 40$ has an efficiency of more than 500000 theoretical plates in 10 min run time.

Design lim 01: S.I. 5; Sep.Ch.W. 10, EVvap.Ch.W.10 by Nils Goedecke 05.07.2000

Fig. 16 (b)

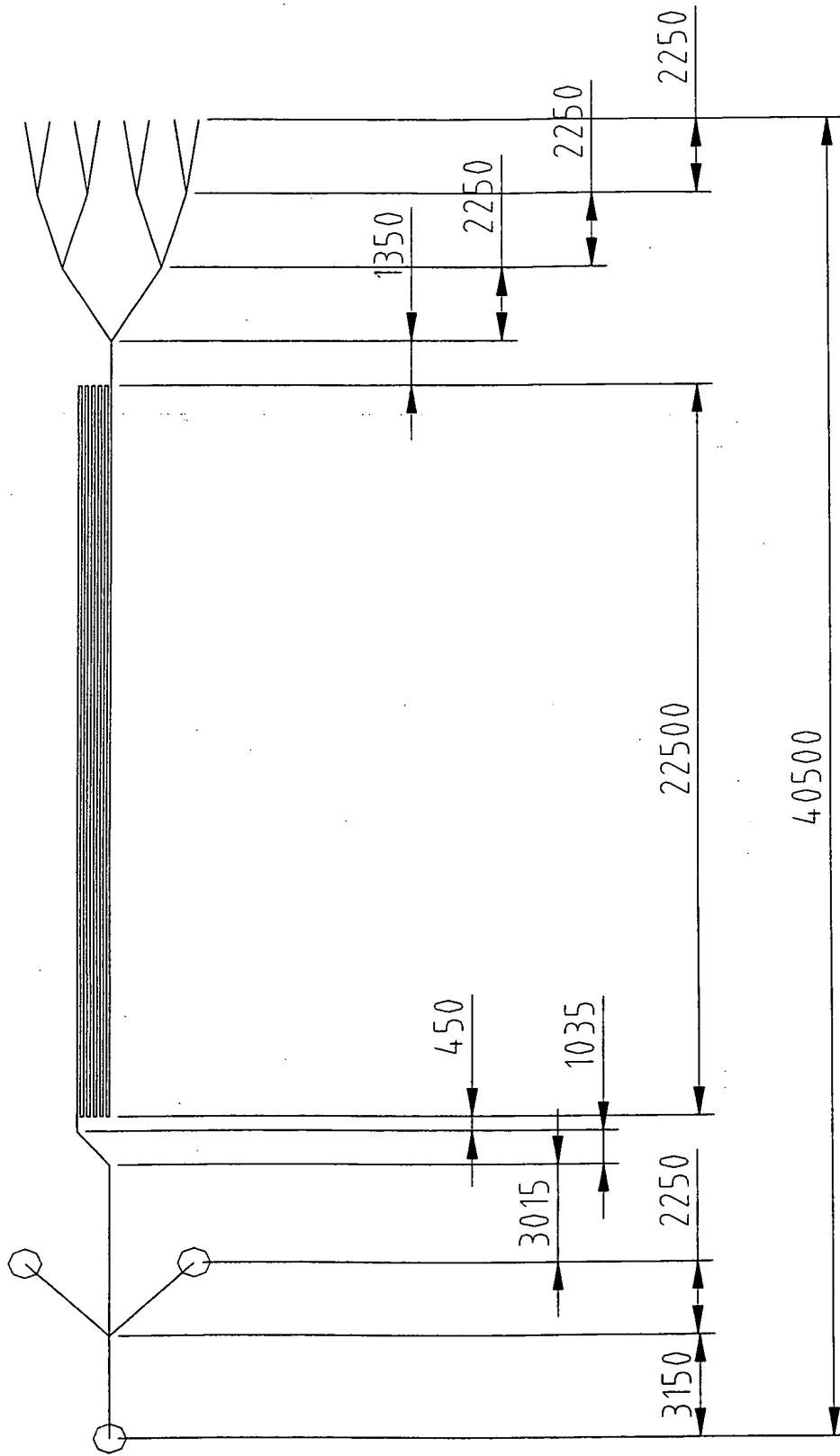
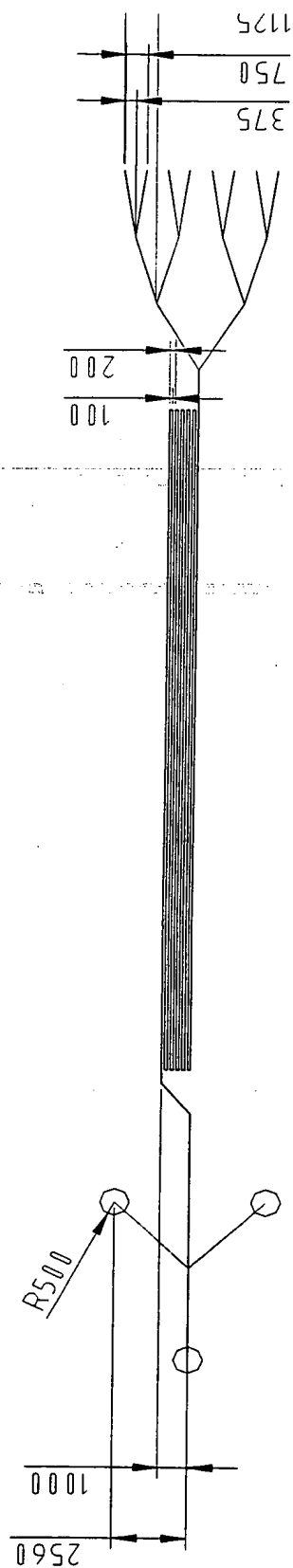


fig. 17(a)

Design lim 02 S.I. 5 Sep Ch.W. 10 EV vap Ch.W. 10 by Nils Goedecke 09.11.2000



Design lim 02 S.I. 5 Sep.Ch.W. 10 EVvap.Ch.W.10 by Nils Goedecke 09.11.2000

Fig. 17(b)

a). Design structure
using CAD package
and convert to
machine format



b). Expose
photoresist
using DWLII
system



HeCd
Laser
 $\lambda=442\text{nm}$

Photoresist

Metal Layer
(Cr or Cr and Au)

Glass Substrate



c). Develop
photoresist



d). Etch Metal
Layer



e). Etch Glass

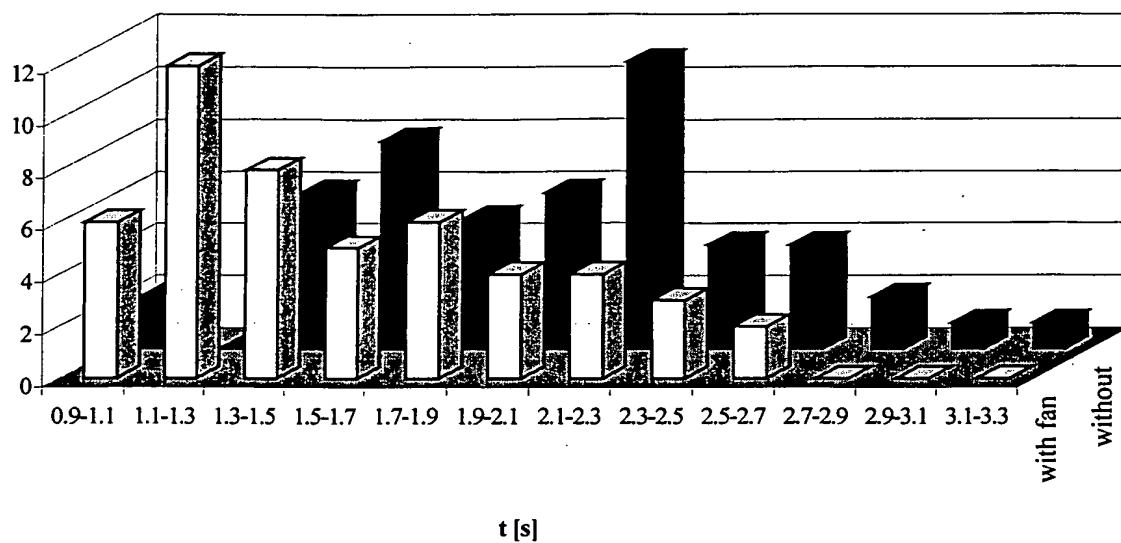


f). Remove Photoresist
and Metal Layer
Thermally bond to
coverplate



Fig. 18

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.



Velocity differences within the channel ($60 \times 20 \mu\text{m}$) for $10 \mu\text{m}$ latex beads in a pop02 chip driven through evaporation with and without "air condition"; measurement with 50 beads each; The average velocity with the "air condition" switched on is slightly higher than without it – visible in the left shift of the profile.

Fig. 19

